Claim Rej ctions - 35 U.S.C. 103

Claims 1-12 and 19-22 are rejected under 35 U.S.C. 103(a) as being obvious over certain alleged statements of prior art in combination with Litzinger itself or in further combination with Maccarone and/or Eastman.

It has been alleged that the use of cationic liposomes for the delivery of polynucleotides known in the art would have been obvious since the prior art shows that oligonucleotides are able to efficiently transfect when complexed with cationic liposomes. It is further alleged that the issue is not whether polydeoxyribonucleotides have the classical transfection ability, but whether the cationic liposomes taught are able to deliver, as vectors, the claimed art known polydeoxyribonucleotides.

Applicants respectfully disagree. The present invention in a certain embodiment is concerned with a pharmaceutical formulation for parenteral administration containing complexes of cationic liposomes constituted of phospholipids and polydeoxyribonucleotides having a molecular weight in the range 15,000-60,000 Da. The polydeoxyribonucleotides are obtained by depolymerization of nucleic acids, and in the complexes the polydeoxyribonucleotides are located on the outer surface of the liposome.

Applicants point out that the technical problem addressed by this embodiment of the present invention relates to complexes of polydeoxyribonucleotides having a molecular weight in the range of 15,000 to 60,0000 Da with cationic liposomes that, as compared to prior art known liposomes with polydeoxyribonucleotides, are stable in water. Stability in aqueous solution allows the preparation of drugs to be used in human therapy, wherein the advantage arising from the complexation with liposomes, that is, the increase of pharmacological activity of polydeoxyribonucleotides in the complexes, can be fully utilized.

No such invention is taught or suggested in the prior art including the cited references. For example, Applicants respectfully point out that the improved stability of the liposome complexes of the present invention can be seen in the application at Tables I-III, where it is demonstrated that as compared to prior art liposome-polydeoxyribonucleotide complexes wherein the same polydeoxyribonucleotide is contained within the liposome, the complexes of the present invention retained a remarkable stability over the 30 day time period considered. In contrast to such improved stability of the present invention, the pharmaceutical preparations of liposome complexes of the prior art lose at least 70% of their activity over the same period of time.

Applicants also wish to again emphasize that the known polydeoxyribonucleotides used in the cationic liposome complexes of the invention are not vectors for genetic information. In other words, the polydeoxyribonucleotides lack mutagenic properties, that is, the ability to transfect genetic material from donor to recipient. Applicants provided evidence on this issue with the filing of January 22, 2002, but submit herein additional evidence on this point. Reviewing the enclosure Informations Pharmaceutique OMS, vol. 1, n.4 1987 (Suppl. to vol. 35, n.5 1981), it can be seen that defibrotide (INN), referred to in the specification (See e.g., page 7, lines 10-14), designates polydeoxyribonucleotides from the lungs or other animal organs, having a molecular weight between 15,000 and 30,000. Such molecular weight is the range of molecular weight of the present invention as claimed.

Reviewing pages 50-52 of the Expert Report on the Toxico-pharmacological Documentation of Defibrotide (February 1994), concerning the properties of polydeoxyribonucleotide as a vector for genetic information, the following can be determined. Defibrotide does not have mutagenic potential in the bacterial system used for

the assay (para. 4.4.1 at page 50). Defibrotide also fails to show any mutagenic potential in yeast cells, when mice serve as hosts (para. 4.4.3 at page 50). Furthermore, in an *in vivo* mutation test procedure, defibrotide does not show any mutagenic potential when administered by intraperitoneal route (para. 4.4.3 at page 51).

It should therefore be quite clear to those of ordinary skill in the art that the compounds of the present invention do not possess transfection capability. Applicants urge that those of ordinary skill in the art would find in the cited references no teaching or suggestion regarding improvements in complex stability with polydeoxyribonucleotides not directed to transfection. It is also to be noted that the technical problem addressed by the present invention is not simply the use of cationic liposomes for the delivery of art known polynucleotides. The present invention is concerned with improving the stability of complexes in aqueous solution.

Moreover, regarding the allegation that the term transfection is used to refer to the transfer of therapeutic molecules other than nucleic acids, Applicants respectfully point out that the reference Lee (U.S. Patent No. 5,908,777), also referenced in the Office Action, appears to contradict such allegation. Lee states that genetic therapy requires methodology for delivering nucleic acids to cell or organism. The method of delivery can be diverse and may include the use of liposomes (col. 1, lines 1-2). Lee further states that non-viral vectors such as liposomes have attracted attention as vehicles for nucleic acids delivery in gene therapy. Cationic liposomes have been the most studied due to their effectiveness in mediating mammalian cell transfection *in vitro* (col. 1, lines 13-20). Lee also explains the mechanism of such transfection: the lipid/nucleic acid complex fuses or otherwise disrupts the plasma or endosomal membranes and efficiently transfers the nucleic acid into the cells (col. 1, lines 24-25). It therefore appears that the cited Lee

reference supports the meaning of the term "transfection" as given in the *Glossary of Biotechnological Terms* submitted with the response of January 22, 2002. That is, the term "transfection" refers to the transfer of genetic material from donor to recipient.

Lee also supports the repeated statements of Applicants that the prior art complexes between DNA and cationic liposomes are know to be inherently unstable. For example, Lee states:

> [T]he DNA/liposome complex is unstable, i.e., it undergoes slow aggregation. The aggregation increase at high DNA concentration, which is required for clinical applications. Therefore, DNA/cationic liposome complexes need to be prepared fresh, which reflects in increased cost and decreased convenience.

(col. 1, lines 56-60). Of course, Tables I-III of the application clearly demonstrate that pharmaceutical preparations of liposome complexes of the present invention are far more stable than those of the prior art. Accordingly, in that the Lee reference confirms both the meaning of "transfection" proposed by Applicants as well as instability of prior art complexes as compared to the present invention, Applicants urge that the present invention as claimed should be considered patentable.

Applicants therefore wish to further emphasize that it appears the technical problem addressed by the present invention has not been fully considered. Applicants again respectfully point out that the present invention is concerned with polydeoxyribonucleotide/liposome complexes with such improved stability as to be useful in preparing stable dosage forms for human therapy. Indeed, as taught in the Lee reference, the traditional instability of nucleic acids with cationic liposome complexes is quite well known in the art. Nevertheless, Applicants have now demonstrated, in particular via the examples of the application, that complexes of polydeoxyribonucleotides having a

molecular weight ranging from 15,000 to 60,000 Da as claimed are quite stable in aqueous solutions. Such stability can only be considered surprising and unexpected in view of the teachings of the prior art including the Lee reference.

Finally, regarding the stability of oligonucleotides, Applicants would like to point out that the Zelphati reference, also referenced in the Office Action, discloses simply a general property of complexes of cationic liposomes with oligonucleotides once delivered to the cells. However, while such complexes may protect the oligonucleotides from nucleases, they could not be used in gene therapy, as they are. The disclosure of the Zelphati reference is concerned with the influence of liposomal complexation on oligonucleotide pharmacokinetics. Indeed, the Zelphati disclosure concerns the half life of a phosphodiester antisense oligonucleotide, which is found to be 15 minutes. However, such disclosure has nothing to do with the technical problem addressed by the present invention, which is not the improvement in the polydeoxyribonucleotide pharmacokinetic profile, but rather pharmaceutical preparations of liposome complexes of polydeoxyribonucleotides with improved stability in aqueous solution, for use even after long periods of time from preparation, without substantial loss of pharmacological activity. Applicants have demonstrated that the pharmaceutical preparations of polydeoxyribonucleotide liposome complexes of the present invention are stable over long periods of time. In contrast, the liposome complex made of the same two components, but wherein the liposomes encapsulate the polydeoxyribonucleotides, has been found unstable. Zelphati simply contains no teaching or suggestion that the pharmaceutical preparations of liposome complexes with oligonucleotides can be stable as in the present invention. Accordingly, the improvements of the present invention must be considered quite unexpected, especially considering the prior art knowledge that cationic liposome-

DNA complexes are inherently unstable and thus should be freshly prepared before administration (col. 1, lines 55-60 of Lee), thereby resulting in increased cost and decreased convenience for human therapy. Applicants therefore urge withdrawal of all rejections.

In view of the amendments and remarks above, Applicants respectfully submit that the application is in condition for allowance and request favorable action thereon.

In the event this paper is not considered to be timely filed, Applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 01-2300. The Commissioner is hereby authorized to charge any fee deficiency or credit any overpayment associated with this communication to Deposit Account No. 01-2300, referencing Attorney Docket No. 108907-09014.

Respectfully submitted,

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Enclosures: Informations Pharmaceutique OMS, Vol. 1, n.4 1987 (Suppl. to Vol. 35, n.5 1981)
Expert Report on the Toxico-pharmacological Documentation of Defibrotide February 1994